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Leaf Production Responses of Lampeni (*Ardisia humilis* Vahl.) to Types and Rates of Manure Application

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Abstract

Ardisia humilis (Myrsinaceae), known as Lampeni (in Indonesia), is part of the wild flora in Southeast Asia and is in some countries. The leaf has medicinal properties for some diseases. The leaves and fruit of this plant are believed to be a treatment for tuberculosis, lung disease, hepatitis, chronic bronchitis, and menstrual irregularities in women. Research into the cultivation of Lampeni is needed to support its usage in Indonesia. The objectives of the research reported here were to find the types and rates of manure to promote leaf production. An experiment was carried out at IPB Experimental Station, Cikarawang Dramaga, Bogor, Indonesia, from December to June 2016. The experiment was laid out in a split plot design with manure types (chicken and cow manure) as main plots and application rates (0, 5, 10, and 15 $t.ha^{-1}$) as sub-plots. The results of the experiment showed that vegetative growth increased with manure application. Total branch number, LAI, leaf fresh and dry weight were higher with 15 $t.ha^{-1}$ chicken manure or 5 $t.ha^{-1}$ cow manure application. Leaf analysis showed that pigment concentration responded negatively to increasing manure application and the highest pigment concentration was found on the plants without manure application.

Keywords: fertilization, leaf pigment, medicinal plant, organic cultivation, Southeast Asian wild flora

Introduction

Lampeni (*Ardisia humilis* Vahl., Myrsinaceae) is a wild plant species that is commonly found in coastal areas, forests, or rural areas, up to 500 m above sea level (asl). In Indonesia it was found in Sumatra, Java, Sulawesi and the Moluccas. Several other Ardisia species have been confused with *A. humilis*. This makes it difficult to interpret the literature and to deduce the exact area of distribution. Lampeni is a shrub with a height of 5-10 m, with ovate leaves (6.5 to 16.5 cm × 2.5-7.5 cm), umbellate inflorescences, and reddish purple fruit with a diameter of 0.6-0.8 cm. Ardisia comprises almost 400 species and shows a pantropical distribution. It occurs throughout South-East Asia with western Malesia as the main centre of diversity. Forty-eight species of lampeni have been recorded in southern Vietnam, Thailand has 72 species, Peninsular Malaysia 75, Borneo 92, and New Guinea 31 (PROSEA, 2003).

A. humilis has been utilized in many countries around the world as ornamental plants, fuel (wood), fruit, and as a traditional medicinal plant (CABI, 2013). The leaves and fruit of many members of the Myrsinaceae family are used as traditional medicines. In West Java, Lampeni leaves are used to treat skin diseases and the fruit is used as an anthelmintic and heart tonic (PROSEA, 2003). Additionally, it is reported that the leaves and fruit of this plant can treat tuberculosis, lung disease, hepatitis, chronic bronchitis, and menstrual irregularities in women (Kobayashi and Mejia, 2005). Lim (2012) stated that this plant has phytochemical activities that are beneficial to human health such as anticancer, antiviral, antibacterial, and anti plasmoidal activities.

Judging from the number of its potential pharmacological functions, Lampeni could be developed as a cultivated medicinal plant. Most species of medicinal plants in Indonesia originate from the natural environment, such as the forests. Cultivation of this traditional medicinal plant would make it easy to harvest its bioactive compounds (Hamzari, 2008).

Fertilization could be one of the cultivation methods used to improve the harvest of Lampeni leaves since nutrient applications can support plant growth and leaf production. According to the Indonesian Ministry of Trade (2014), there are general and specific requirements for herbal medicinal products that require them to be produced organically, one of which

is consumers' demand. Organic food is defined as organically produce product from farm which applies organic practices that include using manure. Ibrahim et al. (2013) stated that the use of organic fertilizer on medicinal plants showed an increase in production of secondary metabolites and antioxidant activity when compared to using NPK 15:15:15 fertilizer. On waterleaf (*Talinum tirangulare*), Susanti et al. (2008) found that bioactive compounds in waterleaf decreased with increasing rates of organic fertilizer. Toor et al. (2006) on tomatoes production found that the mean of plant shoot biomass was significantly higher for the plants grown with mineral nutrient solutions compared with chicken manure and grass-clover. However, the mean total phenolic and ascorbic acid content of tomatoes grown using chicken manure and grass-clover mulch was 17.6% and 29% higher, respectively, than the tomatoes grown with mineral nutrient solutions. Different results found on different species in using organic fertilization, so there is a need to determine the -type and rate of organic fertilizer for the cultivation of Lampeni.

Materials and Methods

The research was carried out at IPB Cikarawang organic experimental station, 1060 48 'EL and 6026' SL. The experiment was conducted in December 2015 and June 2016. One-year-old Lampeni seedlings were grown in the open area using rice charcoal as media, and chicken, and cow manure as treatment. Lampeni is a shade tolerant tropical plant that needs high humidity but without inundation (CABI, 2013). The experiment was conducted in the rainy season from January to June 2016 with rainfall recorded over the period of 415.0, 610.0, 644.0, 558.2, 329.7, and 373.0 mm per month, respectively (BMKG, 2016).
The experiment was laid out in a split plot design with two factors; the main plot is manure types (chicken and cow manure) and the sub-plots are fertilizer rates (0, 5, 10, and 15 t ha^{-1}). Topping was conducted at 10 weeks after planting (WAP). Growth variables including plant height, branch number, stem diameter 5 cm above ground were measured every week and leaf number at 2-16 WAP. Leaf area was measured from 10 fully expanded leaves using gravimetric method. Leaf pigment content including chlorophyll a, chlorophyll b, total chlorophyll, anthocyanin content, and carotenoid were measured in young, fully expanded and old leaves using Sims and Gamon method (2012) with UV-VIS 1280 Spectrophotometer (Shimadzu); total N analyzed with Kjehldahl Method, P with UV-VIS 1280 Spectrophotometer (Shimadzu), and K with Atomic Absorption Spectrophotometry (AAS). Data were analyzed using the F test, followed by 5% Duncan Multiple Range Test (DMRT).

Results and Discussion

The results of the experiment showed interaction of type and manure rates affected leaf area index (LAI) ($P<0.05$) at 17, 19, and 21 WAP (Table 4), branch number 16 WAP (Table 1), stem diameter 22 and 24 WAP (Table 2), and leaf number 18, 20, 22, and 24 WAP (table 3). Leaf fresh and dry weight per plant, which were measured at the end of the observation period, were affected by different types and fertilizer rates ($P<0.05$) (Table 3). Manure that takes time to decompose in the soil possibly caused slow availability of nutrients to the plant that resulted in slow responses (Wu et al., 2017). Results of the analysis of leaf pigment showed young leaf chlorophyll a and b were affected by manure rates; total chlorophyll of the fully expanded and mature leaf by manure types, and young leaf anthocyanin by types and manure rates interaction ($P<0.05$)

Table 1. Branch number and branch number increment with types and manure rates

Manure types	Manure rates (t.ha^{-1})	Branch number (WAP)		Increment in branch number WAP)	
		16	24	2 – 8	10 – 24
Chicken	0	4.0 b	11.0	0.5	8.0
	5	6.3 ab	9.5	1.0	11.0
	10	11.0 a	14.5	1.5	14.8
	15	10.5 a	22.0	5.5	17.8
Cow	0	6.3 ab	21.0	1.5	5.8
	5	11.0 a	12.8	4.5	17.0
	10	6.3 ab	25.0	1.0	11.5
	15	7.0 ab	25.0	2.3	11.8

Note: Values followed with different letters within the same column were significantly different according to DMRT at α=0.05

Branch Number

The results showed that application of chicken manure at 10 t.ha^{-1} increased branch number by 175% compared with no chicken manure, and cow manure application at 10 t.ha^{-1} increased branch number 62.9% compared to without cow manure application (Table 1). Branch number is one of the leaf production components, since all leaves grow on the branches. Cholid et al. (2007) study demonstrated a higher number of branches in the pruned Jatropha compared to the unpruned; pruning stimulates the growth of new shoots.

Stem Diameter

Application of cow manure 5 t.ha^{-1} increased stem diameter 57% compared to no cow manure application at 20 WAP (Table 2).

Leaf Number

At 24 WAP application of chicken manure 15 t.ha^{-1} increased leaf number 157% compared to without chicken manure (Table 3). Qureshi et al. (2014) stated that farm yard manure increased kale (*Brassica oleraceae* "Acephala" yield. In this study, increase chicken manure application increased leaf number, but the application of cow manure decreased it.

Leaf Area Index (LAI)

Table 4 showed that the application of chicken manure at 15 t.ha^{-1} increased leaf area index by 264% compared to plants with no chicken manure application; this might be related to the increase in leaf number in the chicken manure-treated plants as described in Table 3.

Leaf Fresh and Dry Weight

Table 5 shows the values of the leaf fresh weight (g per plant), leaf dry weight (g per plant). Leaf fresh and dry weight were affected with the application of types and manure rates ($P<0.05$). Leaf fresh weight per plant applied with chicken manures at 15 t.ha^{-1} produced 153% higher than without chicken manure. Application of cow manure 5 t.ha^{-1} produced 180% higher than without cow manure. Plants with chicken

Table 2. Lampeni stem diameter and stem diameter increment with types and manure rates

Manure types	Manure rates (t.ha^{-1})	Stem diameter (mm)		Increment in stem diameter (WAP)
		22 WAP	24 WAP	2 – 24 WAP
Chicken	0	1.09 ab	1.22 ab	0.33
	5	0.92 b	0.98 b	0.26
	10	1.42 ab	1.42 ab	0.61
	15	1.56 ab	1.56 ab	0.62
Cow	0	0.93 ab	1.01 ab	0.21
	5	1.39 a	1.59 a	0.66
	10	1.02 ab	1.07 ab	0.28
	15	0.98 ab	1.05 ab	0.21

Note: Values followed with different letters within the same column were significantly different according to DMRT at α=0.05

Table 3. Lampeni leaf number and leaf number increment with types and manure rates

Manure types	Manure rates (t.ha^{-1})	Leaf number increment		Leaf number
		2 – 8 WAP	10 – 24 WAP	24 WAP
Chicken	0	37.0	102.0	130.3
	5	47.0	128.0	128.0
	10	42.8	207.3	149.5
	15	49.5	270.8	291.3
Cow	0	30.8	94.3	263.8
	5	59.3	257.0	197.0
	10	40.5	166.3	334.8
	15	40.0	100.8	117.0

Note: Values followed with different letters within the same column were significantly different according to DMRT at α=0.05

Table 4. Lampeni leaf area index (LAI) with types and manure rates at four to six weeks after planting

Manure types	Manure rates ($t.ha^{-1}$)	Leaf area index		
		4 WAP	5 WAP	6 WAP
Chicken	0	0.06 b	0.09 b	0.14 b
	5	0.13 ab	0.13 ab	0.19 b
	10	0.24 ab	0.29 ab	0.37 ab
	15	0.28 a	0.33 a	0.51 a
Cow	0	0.14 ab	0.14 ab	0.17 b
	5	0.25 a	0.29 ab	0.40 ab
	10	0.14 ab	0.16 ab	0.24 ab
	15	0.12 ab	0.11 b	0.18 b

Note: Values followed with different letters within the same column were significantly different according to DMRT at α=0.05

manure 15 $t.ha^{-1}$ produced 145% more leaf dry weight than without chicken manure, whereas application of cow manure at 5 $t.ha^{-1}$ produced 163% more than without cow manure.

Leaf fresh and dry weight were affected by the leaf number; the largest leaf production was with application of chicken manure 15 $t.ha^{-1}$, or the cow 5 $t.ha^{-1}$ ($P<0.05$). The largest leaf production was also supported by the largest branch number with the application of chicken manure 10 or 15 $t.ha^{-1}$, cow manure 5 $t.ha^{-1}$ ($P<0.05$) whereas the tallest plants were those applied with cow manure 15 $t.ha^{-1}$ (Table 5, $P>0.05$).

N, P, and K Leaf

Application of cow manure 5 $t.ha^{-1}$ resulted in the highest total N leaf; the highest P was without cow manure application, and the highest K with 15 $t.ha^{-1}$ chicken manure application (Table 6).

Leaf Pigment

The results showed that the highest leaf chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids found in the mature leaves without fertilizer (Table 7). The highest leaf anthocyanin found in the medium leaf with manure 10 $t.ha^{-1}$. The highest leaf pigment showed that crop without manure application had higher pigment content than with fertilization. Leaf chlorophyll a without fertilization was 87% higher than with manure application 10 $t.ha^{-1}$. Leaf chlorophyll b without manure application was113% higher than manure application 10 $t.ha^{-1}$. Leaf total chlorophyll without manure application was 94% higher than manure application 10 $t.ha^{-1}$. *Talinum triangulare* applied with organic fertilizer had more shoot fresh weight and flavonoid than those with inorganic fertilizer in dry season (Mualim, 2012). In addition, it had greater dry weight (Theuer, 2015), and more vitamin C (Worthington, 2001; Benbrook et al., 2008; Mualim, 2012).

In *Talinum triangulare* manure application had a higher influence on plant relative growth rate (RGR) and marketable shoots compared to inorganic fertilizer (Aziz et al., 2014). El Gendy et al. (2015) stated that N and/or K fertilizer increased herb and

Table 5. Lampeni leaf fresh and dry weight with types and manure rates

Manure Types	Manure Rates ($t.ha^{-1}$)	Leaf weight (g per plant)	
		Leaf fresh weight	Leaf dry weight
Chicken	0	138.96 c	36.67 c
	5	165.12 c	42.33 c
	10	311.28 ab	83.35 ab
	15	352.09 a	89.86 a
Cow	0	134.14 c	37.92 c
	5	376.88 a	99.67 a
	10	213.55 bc	54.37 bc
	15	123.92 c	31.72 c

Note: Values followed with different letters within the same column were significantly different according to DMRT at α=0.05

Table 6. Lampeni leaf N, P, and K

Manure types	Manure rates (t.ha^{-1})	Nutrient (%)		
		N Total	P	K
Chicken	0	1.71	0.14	2.21
	5	1.74	0.12	3.39
	10	1.57	0.12	3.36
	15	1.68	0.15	3.80
Cow	0	1.78	0.20	1.33
	5	1.83	0.14	1.52
	10	1.65	0.13	2.20
	15	1.69	0.15	3.41

Table 7. Lampeni leaf pigment at different types and rates of manure

	Young leaf		Fully expanded leaf		Mature leaf	
	Chlorophyll a/ chlorophyll b (µg.g^{-1})					
Manure types						
Chicken	573.6	155.20	1,070.8	447.14	1,453.7	616.78
Cow	444.6	221.92	1,349.1	560.53	1,395.7	613.42
Manure rates (t.ha^{-1})						
0	654.3 a	262.24 a	1,391.6	573.65	1,508.7	644.90
5	470.6 ab	173.83 ab	1,218.9	524.69	1,471.3	639.48
10	348.2 b	123.06 b	1,098.1	478.12	1,410.2	559.38
15	563.2 ab	206.24 ab	1,131.1	457.79	1,410.2	639.48
	Anthocyanin/ Carotenoid (µg.g^{-1})					
Manure types						
Chicken	851.6	469.8	1,172.03	948.14	1,107.87	1,171.45
Cow	1,024.1	582.1	1,143.99	1,076.99	1,237.68	1,154.48
Manure rates (t.ha^{-1})						
0	1,092.2	637.85	1,029.8	1,116.0	1,160.70	1,194.9
5	941.7	513.10	1,192.1	1,035.2	1,140.24	1,189.6
10	789.0	416.81	1,255.5	964.2	1,241.35	1,165.0
15	951.3	554.66	1,150.0	956.4	1,170.43	1,099.5
	Total chlorophyll (µg.g^{-1})					
Manure types						
Chicken	602.0		1,607.62 b		2,181.39 a	
Cow	798.8		1,829.38 a		1,897.97 b	
Manure rates (t.ha^{-1})						
0	866.0 a		2,023.1		2,025.0	
5	691.6 ab		1,555.8		1,927.6	
10	445.9 b		1,533.7		1,965.9	
15	798.2 ab		1,761.4		2,240.2	

Note: Values followed with different letters within the same column were significantly different according to DMRT at α=0.05

essential oil yield as well as photosynthetic pigments comparing with control on medicinal and aromatic plants including *Anthriscus cerefolium* L.

Conclusion

The result of the experiment showed that lampeni vegetative growth increased with manure application. Total branch number, LAI, leaf fresh and dry weight were higher with 10 $t.ha^{-1}$ chicken manure or 5 $t.ha^{-1}$ cow manure application. Leaf analysis showed that pigment concentrations respond negatively with increasing manure application; the highest pigment concentrations were found in the plants without manure application.

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